

THE OBLIGATE COMMENSAL CILIATES OF STRONGYLOCENTROTUS DRÖBACHIENSIS: OCCURRENCE AND DIVISION IN URCHINS OF DIVERSE AGES; SURVIVAL IN SEA WATER IN RELATION TO INFECTIVITY

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Seven species of ciliated protozoa have been reported from the alimentary tract of the sea urchin *Strongylocentrotus dröbachiensis* (O. F. Müller) in the coastal waters of Mt. Desert Island, Maine (Powers, 1933a). Three of them are holotrichs which have no known free-living congeners and are restricted to echinoid hosts. They are *Entodiscus borealis* (Hentschel, 1924) Madsen, 1931; *Madsenia indomita* (Madsen, 1931) Kahl, 1934; and *Biggaria gracilis* (Powers, 1933) Kahl, 1934. In the words of Kirby (1941, p. 921), such ciliates "may be supposed to have evolved in the shelter of these hosts" and they are thus regarded as obligate commensals. The relation of the remaining four to their host is not entirely clear, owing to inadequate study. Powers (1933a, p. 119) regards them "as chance or vagrant ciliates, which, after being engulfed with food, are able to survive" and multiply as entozoic commensals. Two of the four are holotrichs, namely, *Plagiopyla minuta* Powers, 1933, and *Cyclidium stercoris* Powers, 1935; one is a hypotrich which Beers (1954) identified as *Euplotes balteatus* (Dujardin, 1841) Kahl, 1932, and the final and least common is an undetermined species of the peritrich *Trichodina*. Reference may be made to Beers (1948) for further details concerning the taxonomy of the ciliates. In order to avoid the constant repetition of the unwieldy binomial *Strongylocentrotus dröbachiensis*, the terms "urchin" and "urchins" are substituted in the following pages and refer without exception to this echinoid.

To return to the three obligate commensals, which are the subject of the present study, Power (1933a) notes that adult urchins at Mt. Desert Island are almost invariably infected with them and indeed may harbor them in almost incredible abundance. In the summer of 1947, Beers (1948) extended Powers' investigations by making a quantitative study of the occurrence and morphogenetic condition of the ciliates in 182 urchins, the tests of which varied in diameter from 30 to 60 mm. All the urchins were infected with *E. borealis* and *M. indomita*, and 181 of them with *B. gracilis*. Counts of the ciliates in fresh samples of enteric fluid showed that the vast majority of the urchins harbored infections of each species that varied in intensity from "moderate" (M) to "heavy" (H), M meaning 50-500 individuals of the species per 0.1 ml. of fluid and H meaning 500-1000 or more per 0.1 ml. The remaining infections were designated as "light" (L), meaning fewer than 50 individuals of a species per sample. A regional distribution of the ciliates was also

noted, in that *E. borealis* occurred primarily in the stomach (inferior spiral or intestine of some authors), *M. indomita* in the intestine (superior spiral or large intestine), and *B. gracilis* in the rectum. However, the foregoing distribution of *E. borealis* and *M. indomita* prevailed as a rule only in well-fed urchins; in inadequately fed urchins they tended to shift aborally and in extreme cases of hunger to commingle with *B. gracilis*. The factors that were responsible for the regional distribution of the ciliates were unexplained.

In any flourishing population of ciliates, whether free-living or associated in any way with a host, one might reasonably expect to find at almost any time a significant percentage of individuals that are dividing. It is therefore remarkable that dividing specimens of *E. borealis* and *M. indomita* are extremely rare, even in ciliate populations of great density. With reference to the division of *E. borealis*, Powers (1933b, p. 130) comments as follows: "A study of about 600 specimens fixed during the day gave but three individuals showing any signs of fission." In 1947 the writer made a special effort to find dividing specimens of *E. borealis* and *M. indomita* in the 182 urchins that have been mentioned. The urchins were collected and examined without delay at practically all hours of the day and night, but only six of them revealed dividing individuals of *E. borealis*. Concerning *M. indomita*, neither Madsen (1931) nor Powers (1933a) mentioned its division, and the task of finding dividing specimens was especially difficult. In the 88 urchins that were examined in July, only one dividing individual was found; in 94 studied in August, dividing specimens were found in only three. It was concluded that division in *E. borealis* and *M. indomita* was a periodic phenomenon: that long intervals of non-divisional life alternated with brief periods of intense divisional activity. In retrospect it became apparent that both Powers and the writer, in their efforts to find dividing ciliates, inadvertently restricted their studies to relatively large mature urchins, in which the ciliate populations were already well-established and probably somewhat stabilized. It will be seen in the following pages that when some of the younger urchins are examined, divisional stages can be found in abundance. Turning finally to *B. gracilis*, it was evident that this ciliate differed markedly in its reproductive activities from the preceding two. Of the 181 infected urchins, all contained dividing specimens, and there was thus no evidence of long periods of non-divisional life.

The relation of the size of the urchins to the condition of their respective ciliate infections was not considered in the earlier study (Beers, 1948). Actually, there is often great diversity of size in an aggregation of urchins on a rocky ledge or in a tide pool. For example, urchins taken by the writer from a single tide pool at Long Ledge, Mt. Desert Island, on July 10, 1960, varied in diameter from 8 to 65 mm. To some extent these differences merely reflected different rates of growth, but Grieg (1928) concluded that size (diameter of test) is a fairly reliable measure of the age of the urchins. Basing his studies on urchins taken from the Folden Fjord and the Bals Fjord of Norway, and on other materials, he concluded that the following relations of size to age prevail, at least in a general way: diameter 0.5 mm., metamorphosis just completed; 1-2.5 mm., "the same year-group" as the foregoing, meaning urchins in their first summer of life; 5-6 mm., about 1 year old; 15 mm., about 2 years old; 24 mm., 3 years old; 40 mm., 4 years; 50 mm., 5 years; 60 mm., 6 years; 78 mm. (the largest specimen), "probably about 8 years

old." In the region of Mt. Desert Island, the spawning of urchins begins in February and ends in April. Since the urchins of northern Europe have a similar spawning period (Mortensen, 1943, p. 211), there is little doubt that Grieg's estimates of age are equally applicable to Mt. Desert Island urchins.

The present paper is a record of further observations on *E. borealis*, *M. indomita* and *B. gracilis* as found in 152 urchins taken at Mt. Desert Island in the summer of 1960. It is based on seven collections or small populations of urchins, each of which consisted of specimens of as many different sizes (age-groups) as were available at the respective sites of collection. The study concerns in particular the following aspects of the biology of the ciliates.

(1) Their occurrence and morphogenetic condition (whether dividing or not) in "small" urchins, meaning urchins 8–14 mm. in diameter and presumably about 1.5 years old. (A minimal size of 8 mm. was fixed solely by the unavailability of any urchins of smaller size.) This aspect attempts to answer these questions: At what age do urchins become infected with the respective ciliates? Once established in the urchin, do the ciliate populations build up immediately or does a delay ensue following their ingestion by the host?

(2) Their occurrence and morphogenetic condition in "larger" urchins, meaning urchins 15–65 mm. in diameter and representing five age-groups, namely, 2.5 to 6.5 years, in increments of one year. This aspect attempts to answer these questions: Do the infections become progressively more intense (ciliates more plentiful) as the urchins increase in age? Is the division of the ciliates, in particular that of *E. borealis* and *M. indomita*, correlated in any way with the age of the urchins?

(3) Their morphogenetic condition throughout a population of urchins. That is to say, does division of the ciliates occur simultaneously in all the urchins of a population or does it affect only certain age-groups or random individuals?

(4) Their capability to survive in sea water outside the body of the urchin, bearing in mind that cysts are unknown in all echinoid ciliates and that young urchins undoubtedly become infected by the ingestion of the usual trophic forms; thus, such survival affects directly their transmission from urchin to urchin.

MATERIAL AND METHODS

Of the seven collections of urchins, three were taken at low tide from the rocks of Emery Cove Ledge on July 2, 13 and 24. The remaining four were taken from four different tide pools at Long Ledge on July 10, August 8 and 26, and September 1. Each collection consisted of about 40 individuals. Of the urchins of each collection, 10 to 15, representing as many age-groups (sizes) as were available, were opened and examined without delay on the day of collection, and a like number was examined on the following day. The remaining ones were excluded from consideration, since it seemed advisable to use only urchins that were relatively recently collected.

The total number of individuals of each species of ciliate was actually counted in the small urchins, but this procedure was usually impracticable with reference to larger urchins, in view of the enormous numbers of ciliates in them. Thus, 0.05-ml. or 0.1-ml. samples of enteric fluid were taken from these urchins, and

the number of ciliates of each species was estimated in the samples. If the size of the urchin permitted, five 0.1-ml. samples were taken from the stomach, five from the intestine, and two from the rectum. In 0.1-ml. samples, the three degrees of infection that have been defined were again distinguished with reference to each species. In 0.05-ml. samples, half the aforementioned numbers of individuals was employed to distinguish the respective degrees of infection.

With reference to the survival of the ciliates in pure sea water, details of the procedure will follow.

RESULTS

1. Occurrence and morphogenetic condition of the ciliates in small urchins (diameter of test, 8-14 mm.)

Unfortunately, only nine urchins of this size were available for study. Nevertheless, it is believed that they furnish information that is significant (Table I).

TABLE I

Total numbers of ciliates of three species in each of nine small urchins (age about 1.5 years) taken at Long Ledge, Mt. Desert Island, in 1960

Diameter of test in mm.	Date collected	<i>Entodiscus borealis</i>	<i>Madsenia indomita</i>	<i>Biggaria gracilis</i>
8	July 10	0	0	0
8	August 26	0	0	0
8	September 1	0	0	0
9	July 10	3	0	1
9	August 26	9	12	2
9	September 1	1	8	0
12	August 8	10	8	1
13	August 26	26	15	4
14	July 10	9	28	2

Since urchins attain a diameter of 5-6 mm. at the end of one year and of 15 mm. at the end of two years, it is assumed that these nine urchins emerged as plutei in February or March of 1959 and were thus about 1.5 years old in the summer of 1960.

A very careful examination of the contents of the digestive tracts of three 8-mm. urchins revealed no ciliates whatsoever, although the digestive tract of each was well filled with algal food. A similar examination of three 9-mm. urchins that were collected on the same dates as the foregoing revealed only small numbers of ciliates, though *M. indomita* was absent in one of them and *B. gracilis* in another. None of the ciliates was dividing. Evidently these three urchins, at the time in their second summer of life, were in the process of acquiring their respective ciliate infections. Finally, an examination of three urchins that had diameters of 12, 13, and 14 mm., respectively, showed ciliates of all three species in each urchin. On the average, these urchins contained two to three times as many individuals of each species as the 9-mm. urchins, even though the infections with *B. gracilis* were extremely light. Again, no dividing specimens were observed.

2. Occurrence and morphogenetic condition of the ciliates in larger urchins (diameter of test, 15-65 mm.)

Urchins 15-23 mm. in diam. Twelve urchins of this size, assumed to be about 2.5 years old and thus in their third summer of life, were available for study. Whereas the urchins of the preceding age-group (1.5 years) were either uninfected or at best only lightly infected, all the urchins of the present group were infected with the three ciliates, and about half of the infections qualified either as moderate or heavy. The status of the respective infections in the 12 urchins was as follows: *E. borealis*, 1 H, 5 M, 6 L; *M. indomita*, 1 H, 4 M, 7 L; *B. gracilis*, 6 M, 6 L. Thus, a marked increase in the intensity of infection was clearly demonstrable in the 2.5-year-old urchins. It is evident that such an increase could have come about either by the ingestion of additional individuals or by the division of those already ingested. Manifestly, no comment can be made concerning the ingestion

TABLE II

Incidence of division of three species of ciliates in urchins of five different age-groups taken at Mt. Desert Island, summer 1960. All the urchins were infected with the three species.

Number of urchins examined	Diameter of test in mm.	Approximate age of urchins in years	Number (and percentage) of urchins that contained dividing ciliates of species indicated		
			<i>Eutodiscus borealis</i>	<i>Madsenia indomita</i>	<i>Biggaria gracilis</i>
12	15-23	2.5	9 (75.0)	7 (58.3)	12 (100)
32	24-39	3.5	2 (6.3)	1 (3.1)	32 (100)
35	40-49	4.5	2 (5.7)	2 (5.7)	35 (100)
37	50-59	5.5	3 (8.1)	3 (8.1)	37 (100)
27	60-65	6.5	2 (7.4)	1 (3.7)	27 (100)

of ciliates during the one-year interim, but it is significant that a remarkably high percentage of the 2.5-year-old urchins contained dividing individuals, showing conclusively that the respective ciliate populations were undergoing rapid augmentation by binary fission. The data concerning division in these urchins are summarized in Line 1 of Table II, reference to which shows that *E. borealis* was dividing in 9 of the 12 urchins, *M. indomita* in 7 of them, and *B. gracilis* in all of them. Furthermore, dividing specimens were relatively abundant, the incidence amounting to about one in every 25-50 individuals of each species. With reference to the division of *E. borealis* and *M. indomita*, it may be said now for purposes of emphasis that in none of the remaining age-groups was there such a high percentage of urchins that contained the two ciliates in division. Concerning *B. gracilis*, it has been pointed out that this ciliate differs in its reproductive activities from the aforementioned two, in that long periods of non-divisional life are absent. Thus, *B. gracilis* was dividing in all 12 urchins.

Urchins 24-39 mm. in diam. Urchins of this size, assumed to be about 3.5 years old, were available in almost unlimited numbers at both collecting sites, as were indeed those of all succeeding age-groups. Of 32 urchins of this size that were examined, all were infected with the three ciliates, as were all the urchins of the age-groups subsequently to be discussed. The respective degrees of infec-

tion among the 32 hosts were as follows: *E. borealis*, 20 H, 10 M, 2 L; *M. indomita*, 18 H, 11 M, 3 L; *B. gracilis*, 7 H, 23 M, 2 L. In terms of percentages, 91 to 94% of the urchins harbored infections of each species that qualified as moderate to heavy. Thus, these urchins were distinctly more heavily infected than those of the preceding two groups, and it will be seen, when older age-groups are considered, that the infections had now attained their maximal intensities.

The findings relative to division are summarized in Table II, Line 2. Of the 32 urchins, only two contained dividing specimens of *E. borealis*, and even in them division was somewhat sparse and affected no more than one specimen in every 100. In spite of an exceptionally thorough examination of the samples, dividing individuals of *M. indomita* could be found in only one of the urchins (a different one from the foregoing two). In accordance with expectations, *B. gracilis* was dividing in all the urchins of the group.

Urchins 40-49 mm. in diam. Thirty-five urchins of this size, assumed to be about 4.5 years old, were examined. The respective degrees of infection follow: *E. borealis*, 15 H, 17 M, 3 L; *M. indomita*, 17 H, 16 M, 2 L; *B. gracilis*, 5 H, 27 M, 3 L. Again, 91 to 94% of the urchins harbored infections that varied from moderate to heavy. Data relative to the occurrence of division are summarized in Table II, Line 3, where it is seen that only two urchins contained dividing specimens of *E. borealis* and a like number (actually another two) those of *M. indomita*. All contained dividing specimens of *B. gracilis*.

Urchins 50-59 mm. in diam. An examination of 37 urchins of this size, assumed to be about 5.5 years old, yielded the following degrees of infection: *E. borealis*, 12 H, 22 M, 3 L; *M. indomita*, 19 H, 16 M, 2 L; *B. gracilis*, 12 H, 21 M, 4 L. With reference to each of the species, 90 to 95% of the urchins had infections that varied in intensity from moderate to heavy. The data concerning the incidence of division, summarized in Table II, Line 4, show that three of the urchins had dividing specimens of *E. borealis* and three (one of the foregoing plus two others) had *M. indomita* in division. As usual, all the urchins contained dividing forms of *B. gracilis*.

Urchins 60-65 mm. in diam. Twenty-seven urchins of this size (age about 6.5 years) were examined. Their respective degrees of infection were the following: *E. borealis*, 13 H, 12 M, 2 L; *M. indomita*, 15 H, 11 M, 1 L; *B. gracilis*, 9 H, 16 M, 2 L. Again, with reference to each species, moderate to high infections comprised more than 90% of the total. Of the 27 urchins, two harbored divisional stages of *E. borealis* and a third one contained *M. indomita* in division (Table II, Line 5), whereas *B. gracilis* was dividing, as expected, in all of them.

3. Morphogenetic condition of the ciliates in the respective collections of urchins

It has been mentioned that the urchins of the present study comprised seven collections, each of which may be regarded as a small population; each at least is believed to be a fairly representative sample of a natural population. And it has been shown, within the limits of the available material, (1) that urchins 8-14 mm. in diameter (age 1.5 years) may or may not be infected, but that if infected, they contain no dividing ciliates (Table I); and (2) that all urchins 15-23 mm. in diameter or larger (2.5 years of age or older) are infected with the ciliates, that *B. gracilis* is constantly dividing in all of them, but that *E. borealis* and *M. indomita*

can be found in division in only a limited, though variable, number of them (Table II). However, the data concerning the division of *E. borealis* and *M. indomita* in certain urchins of ages 2.5–6.5 years, as presented in Table II, tell nothing about the distribution of these particular urchins in the respective collections or population samples. Thus, one may ask: If *E. borealis* and *M. indomita* are dividing in most of the urchins of one age-group of a collection—for example, the 2.5-year group—are they also dividing in a like percentage of urchins of the remaining age-groups of the same collection?

This aspect can be adequately presented by considering in detail the composition of two typical collections of urchins of ages 2.5–6.5 years and the condition of the two ciliates therein. The collections are those taken at Long Ledge on July 10 and August 8. The results are presented in Table III, in which the left column under the headings beginning "No. of urchins" refers to the collection of

TABLE III

Incidence of division of two species of ciliates in two collections of urchins taken at Long Ledge, Mt. Desert Island, on July 10 and August 8, 1960. All the urchins were infected with both ciliates.

No. of urchins examined		Range in size of urchins in mm.	Approximate age of urchins in years	No. of urchins that contained dividing ciliates of species indicated			
				<i>Entodiscus borealis</i>	<i>Madsenia indomita</i>		
5	4	15–23	2.5	4	3	4	3
5	5	24–39	3.5	1	1	0	0
5	5	40–49	4.5	0	0	2	0
5	5	50–59	5.5	2	0	1	1
5	4	60–65	6.5	0	1	1	0

July 10, the right to that of August 8. Line 1 shows that five urchins of the size and age indicated were taken on July 10 and four on August 8. Of the five, four contained *E. borealis* in division and four had *M. indomita* in division. (Three of the five contained dividing individuals of both species.) Of the four urchins taken August 8, three had *E. borealis* and three had *M. indomita* in division. (Two had both species.) If the nine urchins are considered as a group, seven of them or 77.7% contained dividing individuals of *E. borealis* and seven contained *M. indomita* in division.

What was the condition of the two ciliates in the remaining age-groups of the two collections? Was division as widespread in the urchins of these groups? The answer is conclusively in the negative, as shown in the remaining four lines of Table III. For example, Line 2 shows that five 3.5-year-old urchins of each collection were examined. Only one urchin of each collection contained *E. borealis* in division; in none of the ten was *M. indomita* dividing. The urchins of the remaining age-groups revealed essentially similar findings (Lines 3–5). Thus, division in *E. borealis* and *M. indomita*, when it occurs in a population of urchins, does not necessarily affect uniformly all the urchins of the different age-groups of the population.

4. *Survival of the ciliates in sea water and its relation to infectivity*

It has been pointed out that cysts are unknown in ciliates of echinoids and that young hosts undoubtedly acquire their faunules by the ingestion of trophic forms that escape among the fecal pellets. This conclusion implies that echinoid ciliates can live in sea water outside the body of the host, although information on their survival is meager. Powers (1933b, p. 123) states that specimens of *E. borealis* when transferred to sea water "appear normal" and "live for various lengths of time," and he was able to keep specimens in hanging-drop preparations at 7° C for periods that varied from 15 to 23 days. It is doubtful that the survival of a large entozoic ciliate in the restricted confines of a small hanging drop reveals anything of special significance about its survival under natural conditions, and Powers himself states that the animals seemed "merely to exist." Since the capability of echinoid ciliates to survive in sea water is inseparably related to the infection of new hosts, a study of the survival of the three entocommensals of *S. dröbachiensis* was undertaken, but the procedure differed radically from that of Powers.

The sea water was taken from Frenchman Bay (mean annual salinity, 31.8) well beyond the intertidal zone and was passed through Whatman No. 43 filter paper to remove the predatory or unwanted plankters. Each of the three species was dealt with separately in the following manner, as illustrated by *E. borealis*. A clean pre-cooled Syracuse watch glass was placed on the stage of a dissecting binocular and filled with 10 ml. of sea water (temperature 15° C., approximately that of Salisbury Cove sea water in the summer of 1960). Then, about 75 specimens of *E. borealis* from a recently collected urchin were placed in the watch glass near its right margin. The ciliates usually dispersed rapidly, so that many of them soon arrived in relatively pure sea water at the left margin of the watch glass, whereupon 25 of them were transferred by means of a small pipette to 1 ml. of fresh sea water in a Columbia culture dish (square plate-glass depression slide, measuring 42 mm. on a side). The culture dish was placed in a covered Stender dish which was outfitted as a small moist chamber and kept in a tray of running sea water to maintain the temperature at 15° C. The condition of the ciliates was observed and recorded at the end of 6, 9, 24, 48, 72, and 96 hours, reckoning from the beginning. The experiment as just described was repeated some 20 times, using ciliates from more than a dozen different urchins. The method was decidedly superior to the use of hanging-drop preparations, in that the ciliates were first allowed to wash themselves relatively free of intestinal materials and were then transferred to 1 ml. of fresh sea water, which is a relatively large volume for only 25 ciliates. In most of the experiments the final culture dishes were exposed to the natural light of the laboratory, but in some they were kept in darkness (Stender dishes painted black on the outside) except during the brief intervals of observation. Since the histories of the cultures were identical, there was no evidence that moderate illumination was detrimental to the ciliates or that darkness was beneficial.

The procedure that has been described for *E. borealis* was likewise employed with *M. indomita* and *B. gracilis*. To facilitate comparisons, the results obtained with 300 individuals of each species, representing 12 culture-dish experiments, will be considered (Table IV).

E. borealis. Upon transfer to sea water, the ciliates, in agreement with Powers' findings, showed little or no heightened irritability and suffered no observable distortion in shape. At the end of 6 hours, 297 of the original 300 were present in the cultures, and at end of 9 hours, 296. It is likely that the death and disappearance of a few resulted from injuries that accompanied the process of washing and transfer. At the end of 24 hours, 281 were present (survival, 93.7%). Some were swimming normally and others were creeping on the bottom of the dish or against the surface film. However, the many food vacuoles which they originally contained had disappeared, and thus the cytosome was relatively transparent. At the end of 48 hours, 256 (85.3% of the original number) were still present, but they were distinctly smaller, quite transparent, very slow of movement, and evidently much weakened from lack of food. During the succeeding 24-hour period

TABLE IV

Survival of three species of urchin ciliates in sea water. Total number of individuals of each species at beginning of experiment was 300. Hours cited are reckoned from the beginning.

	Ciliate		
	<i>Entodiscus borealis</i>	<i>Madsenia indomita</i>	<i>Biggaria gracilis</i>
No. of survivors after			
6 hours	297	298	217
9 hours	296	298	103
24 hours	281	295	0
48 hours	256	272	
72 hours	17	36	
96 hours	0	0	

the animals suffered drastic mortality, since only 17 (5.7%) remained at the end of 72 hours. These few survivors were much smaller than formerly and were barely able to swim. At the end of 96 hours, there were no survivors.

M. indomita. Unlike *E. borealis*, this ciliate when transferred to sea water displayed greatly heightened irritability, for the animals swam rapidly and erratically. However, their intense activity subsided within 5 to 10 minutes, and with no ill effects, to judge by their survival. In general, the results paralleled those obtained with *E. borealis*, although there were slightly more survivors throughout the first three days. At the end of 24 hours all the food vacuoles had disappeared from the cytoplasm, but the animals were still swimming normally. At the end of 48 hours they were considerably diminished in size and were very transparent, and their locomotion was extremely sluggish. Again, a high mortality occurred during the third 24-hour period, such that only 36 were present after 72 hours. No survivors remained at the end of 96 hours.

B. gracilis. The outcome of the experiments with this ciliate was entirely unexpected. Upon transfer to sea water, *B. gracilis* swam rapidly and quite erratically, as if the medium were distinctly unfavorable. Of the original 300 specimens, only 217 (72.3%) were present after 6 hours, and at the end of 9 hours this number was reduced to 103. Many of these were vacuolated and clearly

abnormal in structure, and the remains of others were visible in the culture dishes. Since nearly all the survivors contained food vacuoles, the many deaths among the animals could not be attributed to starvation, but must have resulted from the properties of the medium. At the end of 24 hours there were no survivors.

DISCUSSION

A comprehensive investigation of the relation of the three ciliates to their host in the Mt. Desert Island region would require a study of urchins of practically all sizes taken during all the months of the year. Unfortunately, such a study has not been feasible, and the present one is admittedly incomplete. Nevertheless, the results are of special interest and are fully adequate, it is believed, to support the conclusions that are advanced in the following sections.

1. Acquisition of infections by young urchins and the delayed onset of division

The absence of ciliates in 8-mm. urchins (age about 1.5 years) indicates that young urchins do not acquire their infections during their first summer of life, or even during the first year. The presence of relatively small numbers of ciliates in urchins 9-14 mm. in diameter (age likewise about 1.5 years, but no doubt somewhat older than the foregoing) indicates that the urchins first acquire their ciliates during their second summer when they are at least 9 mm. in diameter and about 1.5 years old.

It might reasonably be assumed that all urchins would become infected not long after metamorphosis and that all would contain fairly dense populations of ciliates by the middle of their second summer. Actually, at least four factors militate against the early acquisition of infections by young urchins at Mt. Desert Island. The first three are of general occurrence; the fourth is to some extent peculiar to the region of the Island. They are the following. (1) The ciliate losses that accompany the extrusion of fecal pellets are relatively small, to judge by earlier experience (Beers, 1948), as if each ciliate resists dislodgement from its preferred segment of the gut. Thus, urchin ciliates are extremely scarce and very difficult to find in the waters of the urchin's natural habitat, though they can be found with no difficulty in the bottom sediments of an aquarium that is well-stocked with urchins. (2) The period of survival of the ciliates in a healthy condition in sea water outside the body of the host is relatively short, varying from 6 to 48 hours. Although the ciliates tend to adhere loosely to any substratum and to creep upon it, thereby facilitating to some extent their ingestion by a new host, the length of time available for their chance discovery and ingestion by an urchin is distinctly limited. (3) Some of the ciliates are no doubt destroyed by predators. (4) Tidal extremes are great, the mean tidal range being 10.6 ft. (3.23 m.) at Salisbury Cove. Thus, enormous quantities of water ebb and flow twice daily over the urchins and undoubtedly carry away many of the extruded ciliates. In view of the existence of these inimical factors, it is perhaps not so surprising to find that the infection of the young urchins is appreciably delayed.

The absence of dividing ciliates in urchins 9-14 mm. in size suggests that the respective ciliate populations do not undergo augmentation by cell division immediately after the infection of the host, but are increased during the second summer

only by the ingestion of additional specimens. Significant augmentation by division appears to be delayed until the third summer, when the urchins are about 2.5 years old.

2. Establishment of the ciliate populations; division of the ciliates in urchins of diverse ages and in populations of urchins

Whereas none of the infected 1.5-year-old urchins contained dividing ciliates, it has been seen that of 12 infected urchins of age 2.5 years, 9 had *E. borealis* in division, 7 had *M. indomita*, and all had *B. gracilis*. These findings indicate, as has been said, that it is during the third summer of the urchin's life that the respective ciliate populations first experience augmentation by division, resulting in the establishment of populations of maximum density.

Once the populations of *E. borealis* and *M. indomita* are established, infrequent eruptions of divisional activity seem adequate to maintain them in the host; thus, relatively few (3.1 to 8.1%) of the older urchins (age 3.5 to 6.5 years) harbor them in division. The factors that are responsible for the seemingly long intervals of non-divisional life and the occasional, intense outbreaks of division are unexplained, as has been said. It has been seen that division in the two cannot be correlated with the age of the host; it seems to occur randomly in older urchins, irrespective of their age. Neither does their division affect *en masse* the individuals of an urchin population, even though the urchins seem to be living under similar conditions. Various possibilities present themselves by way of explanation, for example: (1) There is an inherent rhythm of long frequency in the reproductive activities of the ciliates. (2) Division is correlated, either qualitatively or quantitatively, with the food of the urchin and with the nature of the intestinal flora. Although sea-weeds are the preferred food of *S. dröbachiensis*, it is actually omnivorous, and the nature of the intestinal contents is somewhat unpredictable. Thus, urchins of a collection from one and the same tide pool at Long Ledge were found to have fed on a variety of materials. Some contained principally filamentous green algae in their alimentary tracts; others, bladder wrack (*Fucus* and the like) and sea lettuce (*Ulva*); still others, calcareous algae; and finally some contained non-descript materials that seemed to consist of barnacle remains and bottom sediments. To what extent these diverse food materials affect the ciliate fauna has not been ascertained. (3) Division is correlated with the physiological state of the urchin, though practically nothing can be said at present concerning this point. Of the foregoing possibilities, the second would seem to be the most readily amenable to experimental analysis, and it is planned that progress in this direction will be attempted within the near future.

The probable significance of the constant and uninterrupted division of *B. gracilis* is mentioned below.

3. Survival of the ciliates in sea water in relation to infectivity

It has been seen that under the conditions of the experiments both *E. borealis* and *M. indomita* can tolerate pure sea water for about 48 hours. Evidently this interval of time, assuming that it also prevails under natural conditions, is adequate to insure the eventual ingestion of a sufficient number of individuals to perpetuate the two species in the host.

Much in contrast with the two-day survival of the foregoing species is the seeming incapability of *B. gracilis* to tolerate sea water longer than 6–12 hours. Although little can be said with certainty in explanation of this peculiarity, certain aspects of the autecology of *B. gracilis* seem worthy of mention. Of all the species of ciliates that occur in *S. dröbachiensis* at Mt. Desert Island, *B. gracilis* is the only one that is primarily an inhabitant of the rectum. In this disadvantageous site, it is expelled in greater numbers than any of its confreres (Beers, 1948). But it is also the only one of the ciliates, with the exception of *Euplotes balteatus*, which is probably nothing more than a facultative commensal (Beers, 1954), that is constantly dividing within the urchin. Thus, its loss in greater numbers is offset by frequent division, and its continued survival in the host is reasonably assured. In sea water outside the body of the host, *B. gracilis* experiences a further disadvantage from the standpoint of survival, in that it has relatively little tolerance for sea water. But it is lost in greater numbers from its host, as has just been said. The escape of larger numbers of individuals into the external world would seem to compensate adequately for the briefer period of survival of each; thus, a relatively constant number of individuals is presumably maintained in the external environment, where they can be ingested by new hosts. Though vulnerable to excessive losses both within the urchin and without, *B. gracilis* nonetheless maintains itself by the agency of constant division.

SUMMARY

1. The first part of the study concerns certain relationships of the ciliates *Entodiscus borealis*, *Maðsenia indomita* and *Biggaria gracilis* to their host, the sea urchin *Strongylocentrotus dröbachiensis*. It is based on an examination of 152 urchins taken at Mt. Desert Island, Maine, in the summer of 1960. The respective ages of the urchins are estimates based on size (diameter of test). The second part concerns the survival of the ciliates in sea water, since their survival is inseparably related to the infection of new hosts.

2. Nine urchins measuring 8–14 mm. in diameter (age 1.5 years) were either uninfected or very lightly infected, and none of the ciliates was dividing. Urchins evidently acquire their ciliates at this age (second summer).

3. All the urchins of the remaining age-groups were infected with all 3 ciliates. Of 12 urchins that measured 15–23 mm. in diameter, all contained dividing specimens of *B. gracilis*, 9 contained dividing individuals of *E. borealis*, and 7 contained *M. indomita* in division. The results indicate that the respective ciliate populations build up rapidly to maximal densities in the third summer of the urchin's life (age about 2.5 years).

4. The remaining urchins were assigned by size to 4 age-groups. The number of urchins in each group, their range in size, and their estimated ages follow: 32 urchins, 24–39 mm., 3.5 years; 35, 40–49 mm., 4.5; 37, 50–59 mm., 5.5; 27, 60–65 mm., 6.5. All the urchins harbored dividing specimens of *B. gracilis*; thus this ciliate remains in constant division once infection is well established. But in each group only a small percentage of the urchins (3 to 8%) contained dividing specimens of *E. borealis* and *M. indomita*. Thus, their division, though evidently cyclical, could not be correlated with the age of the urchins.

5. In a natural population of urchins, the division of *E. borealis* and *M. indomita* does not affect simultaneously any large percentage of the urchins. Except in 2.5-

year-old urchins, it appears to occur randomly. Since the urchins of a population practice dissimilar food habits, it is possible that division is correlated with the nature of the food and the subsequent intestinal flora.

6. In pure sea water most specimens of *E. borealis* and *M. indomita* can survive about 48 hours, and their death is due to starvation. Individuals of *B. gracilis* can survive no longer than 6–12 hours, and death does not result from starvation but seemingly from the properties of the medium. It is suggested that the constant voiding of *B. gracilis* among the fecal pellets of the host compensates for its relatively brief period of survival in sea water.

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